Journal of RAMAN SPECTROSCOPY

Identification of Dye Content in Colored BIC Ballpoint Pen Inks by Raman Spectroscopy and Surface Enhanced Raman Scattering (SERS)

Journal:	*Journal of Raman Spectroscopy*
Manuscript ID	JRS-18-0275.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	15-Oct-2018
Complete List of Authors:	Alyami, Abeer; Nanotechnology Barton, Killian; Cork Institute of Technology, CAPPA Lewis, Liam; Nanotechnology Mirabile, Antonio; Mirabile; Iacopino, Daniela; Nanotechnology
Keywords:	BIC Ballpoint, Raman spectroscopy, SERS, Ag nanopastes, art conservation

Identification of Dye Content in Colored BIC Ballpoint Pen Inks by Raman Spectroscopy and Surface Enhanced Raman Scattering (SERS)

*Abeer Alyami,[a] Killian Barton,[b] Liam Lewis,[b] Antonio Mirabile,[c] Daniela Iacopino[a]**

[a]Tyndall National Institute, University College Cork, Dyke Parade, Cork, Ireland

[b]Centre for Advanced Photonics & Process Analysis (CAPPA), Cork Institute of Technology, Bishoptown, Cork, Ireland

[c]Mirabile, 11 Rue de Bellefond, 75009 Paris 09, France

*corresponding author email: daniela.iacopino@tyndall.ie, ORCID: 0000-0003-2301-9401

Keywords: BIC ballpoint, Raman spectroscopy, SERS, Ag nanopastes, UV-vis, art conservation

Abstract

Raman spectroscopy and Surface Enhanced Raman Scattering (SERS) were used for the elucidation of dye content in commercial BIC ballpoint pens. In contrast to the majority of studies in this field on black and blue inks of forensic interest, this paper targeted characterization of colored (red, pink, purple and green) pen inks, increasingly used for artistic purposes. Because of its not invasive nature, the capabilities of Raman spectroscopy were initially tested. However, overall SERS provided enhanced spectral features and quenching of fluorescence necessary for the unequivocal identification of dye mixtures in all analyzed pens. SERS analysis was carried out *in situ*, by deposition of Ag nanopastes directly on pen colored paper surfaces. Rhodamine B was identified as the main dye component in red, pink and purple inks, while phthalocyanine dye blue 38 was identified in all green inks; excitation at different wavelengths revealed that the darker hue of purple ink was achieved by addition of crystal violet to red rhodamine B and that green hues were achieved by addition of yellow dyes to blue 38. UV-vis spectroscopy and thin

layer chromatography (TLC) analyses complemented Raman/SERS measurements by revealing the presence of additional yellow/orange and blue components in red and green inks, respectively. The relevance of this analysis for art diagnostics was demonstrated through the real non-invasive analysis of BIC pens drawings, which led to successful identification of chemical ink composition and identification of production medium.

1. Introduction

Since their introduction in the market in 1945, ballpoint pens have attracted the interest of artists, fascinated by the possibility of generating novel artistic effects and precision line-work not easily obtainable by brush. The low cost, large assortment of colors and hues available, widespread availability and portability of ballpoint pens are among other qualities that have made ballpoint pens the medium-of-choice for the production of pen- and mixed-media artworks.[1] Many of these artworks are today hosted by museums and private collections all over the world.

Ballpoint pen inks are complex mixtures of several dyes and pigments constituting up to 50% of the total ink formulation contained in either a glycol-based solvent or benzyl alcohol.[2,3] Additional components (vehicle) include fatty acids, softeners and polymeric resins, designed to improve the consistency, flow or drying characteristics of the ink.[4] This complex composition makes the identification of dyes in inks challenging. Additional difficulties are constituted by trademark protection and periodical introduction in the market of novel products with slightly modified formulations. Nevertheless, elucidation of dye chemical composition in commercial inks is of paramount importance for the preservation of ballpoint-based artworks, currently endangered by the fast color fading induced by exposure to light.[5]

Most of the research on commercial pen inks so far has focused on identification of dyes in inks of forensic interests such as blue and black pens, universally used for writing of documents (analysis of questioned documents and forgeries).[6-9] However, recently, research interest has

started to shift towards chemical characterization of commercial pen inks used in contemporary art as shown by Zaffino *et al.*[10] and Alyami *et al.,*[11] who both developed spectroscopic methods for the identification of fountain and ballpoint pen inks, respectively. In parallel, efforts have concentrated on the application of analytical techniques alternative to conventional chromatographic methods, requiring high cost instrumentation and relatively large (mg) amount of material for analysis. In fact, the ideal analytical tool for analysis of artstuff should be low-cost, potentially deployable and minimally invasive, in order to fulfill requirements of *in situ* analysis and analytical object integrity preservation. For this reason spectroscopic techniques such as Fourier transform infrared (FTIR),[12,13] X-ray fluorescence,[14] Raman spectroscopy[15,16] are now preferentially being applied to the analysis of inks. Among these, Raman spectroscopy is increasingly becoming the technique of choice, due to its inherent non-destructive nature, sensitivity, fingerprinting ability and availability of low cost and deployable handheld instrumentation. However, Raman spectroscopy suffers from fluorescence interference and low sensitivity, which for a long time have prevented the efficient characterization of dyes and pigments present in heritage objects. These limitations were finally circumvented in the late 1980's, when Surface Raman Enhanced Scattering (SERS) was successfully applied to the identification of dyes in a variety of artistic media.[17, 18] SERS is a surface-sensitive analytical technique that involves the amplification of Raman signals by several orders of magnitude for molecules adsorbed on metallic nanostructures surfaces.[19-21] The observed high signal enhancements have made SERS particularly amenable to the investigation of artistic materials where mass-limited samples are often available and *in situ* applications and local identification of selective analytes is often required.[22-23] As result, many authors have reported successful investigations of lakes and dyestuffs in a large range of matrices including archaeological textile fibers,[24] paper and woodblock prints.[25] One of the major drawbacks associated to the application

of SERS to the analysis of colorants is its inherent, though minimal, invasive nature, associated to the need for extraction or hydrolysis procedures sometimes not applicable to direct analysis of artworks. Nevertheless, its widespread application for the last 20 years suggests that minimally invasive analytical techniques can be often acceptable if high sensitivity and selectivity are obtained. Moreover, the application of SERS has allowed the construction of comprehensive natural and synthetic colorant spectral databases, which provide support for identification and preservation and establishment of provenance and originality of artworks.[26]

Recently our group has successfully applied Raman and SERS, in combination with bench and handheld instrumentation, for the identification of the major dye constituents of felt tip pens.[27, 28] The application of SERS allowed circumventing fluorescence interference and provided high intensity spectral profiles of fluorescent dyes under visible wavelength illumination.[29]

In this paper, we report on the application of Raman and SERS spectroscopies to the identification of dye mixtures in colored BIC ballpoint pens. Ten BIC ballpoint pens with different hues of green, purple, pink and red were analyzed. Particular emphasis was placed on Raman spectroscopy where the effect of different excitation wavelengths, use of bench *vs* handheld instrumentation and use of Raman *vs* SERS was analyzed in detail for each diagnostic ink. Limitations and merit of both Raman and SERS were highlighted. Data collected were verified and complemented by the use of UV-vis spectroscopy and TLC. Finally, the application of Raman spectroscopy and its capability for ink compositional analysis were discussed in the context of a real case study.

2. Experimental Section

Materials. Tetrachloroauric acid, silver nitrate, Trisodium citrate and MeOH were purchased from Sigma-Aldrich. All glassware was cleaned with *aqua regia* prior to nanopaste synthesis. Milli-Q water (resistivity 418 MOhm cm^{1}) was used throughout the experiments. Reference dyes

rhodamine B, tartrazine, Blue 38 and crystal violet were also purchased from Sigma and used without further purification. Commercial BIC ballpoint pens were purchased from local stores. The list of pen analyzed is shown in **Table 1**.

Synthesis of Ag nanopastes. Ag nanopastes were synthesized according to a modification of the Lee and Meisel method reported by Polavarapu *et al.*[30] Briefly, trisodium citrate solution (4.5 mL, 1.00 wt%) was added to an aqueous boiling solution containing $AgNO_3$ (200 mL, 42 mg) under vigorous stirring. The reaction was boiled for another 1 hr and then cooled to room temperature. The obtained Ag nanoparticles in water (200 mL) were centrifuged at 7000 rpm for 20 min and then redispersed in water (2 mL) to obtain Ag nanopaste (3 mg/mL).

Scanning electron microscopy (SEM) images of nanopastes deposited on SiO_2 substrate and on paper were acquired using a field emission SEM (JSM-6700F, JEOL UK Ltd) operating at beam voltages of 2 kV.

Optical Characterization. UV-vis spectra were acquired using an Agilent/HP 8453 UV-vis Spectrophotometer (200 nm to 1100 nm). Raman spectra at 514 nm were obtained from a Renishaw inVia Raman system. A helium–neon laser was employed as an excitation source. The laser beam was focused onto the sample through a Leica 20X objective with 0.5 N.A. Measured power at the sampling level was controlled at about 0.3 mW. Acquisition time was usually 10 s. Raman spectra at 532 and 785 nm were obtained from a Pelkin Elmer Raman station. The laser beam was focused onto the sample through a 50 objective (MPlan Achromat) with 0.75 N.A. The laser power was around 35mW and typical acquisition time was 10 s. Hand held Raman spectra at 785 nm were obtained from an InPharma spectrometer. The laser power was 50 mW at sample and acquisition time was between 7 and 20 s. To obtain SERS spectra, 2 µl of Ag nanopaste were deposited on pen lines and squares drawn on commercial paper, followed by immediate analysis.

Thin Layer Chromatography. Thin layer chromatography (TLC) analyses were performed using silica plates 10 X 20 cm (Sigma-Aldrich). The BIC ballpoint pens and reference samples were deposited as concentrated MeOH solutions. The TLC plates were developed in a horizontal developing chamber. The solvent systems include: ethylacetate/ethanol/water (7:3.5:3 v/v/v) and 1-butanol/ammonia/ethanol (5:3:2 v/v/v). Chromatographic development of plates was performed at room temperature for 60 min.

3. Results and discussion

The list of pens analyzed in this work is reported in **Table 1**.

Figure 1 shows a photograph of colored squares drawn on paper with BIC pens selected for the analysis. From a visual point of view red Fine and Medium Crystal inks resulted quite similar in color, red Fine Crystal showed a darker red coloration whereas red Medium showed a bright-red lighter coloration. Green Fine and Medium Crystal pens showed similar dark green coloration, green Crystal showed a lime hue coloration and green Medium displayed a green coloration.

Images for other Crystal pen squares are also reported, displaying intense pink and purple colorations.

SERS probes used in this work were constituted by Ag colloidal pastes obtained by modification of the classical Lee and Meisel method described in details in the Experimental Section.[30] The nanopastes were constituted by concentrated aqueous solutions of Ag nanoparticles (by a factor of 2, **Figure S1**), which were deposited directly on colored pen areas drawn on paper. The use of Ag nanopastes instead of traditional diluted Ag colloidal solutions resulted in several advantages: i) allowed direct deposition of nanoparticles on colored papers, eliminating the need for cumbersome multiple colloidal deposition and aggregation steps;[31] enabled *in situ* measurements and allowed avoiding the use of time consuming extraction or separation methods often used in association with Raman/SERS approaches;[32] ii) allowed formation of well-defined and large SERS-active areas with high concentration of hot spots and therefore the generation of sensitive SERS responses.

Figure 2(a) shows an optical microscopy image of a paper sheet with a green line written by Medium Crystal BIC pen. The image clearly shows deposition of green ink on the paper fibers. The bottom right part of the image shows the area where Ag nanopastes were deposited, displaying even deposition of the nanopaste over the green colored paper fibers. The inset of **Figure 2** shows a photograph of the green line on paper with deposited Ag nanoparticles. **Figure 2(b-c)** show different magnification SEM images of the Ag nanopaste deposited on the green line paper. It was clear that the colloids evenly covered large areas of the paper substrate and wrapped uniformly around the paper fibers. Moreover, the high SEM magnification of the deposited colloids showed preservation of their original spherical shape and formation of high density and high uniformity particle areas, requisites both important for the achievement of high intensity and reproducible SERS signals. An analogous figure displaying the low density coverage obtained by

direct droplet deposition of diluted colloidal nanoparticles on colored paper is reported in **Figure S2**.

Raman and SERS measurements were carried out *in situ* on coloured lines drawn on commercial paper with excitation wavelengths of 514 nm (see Experimental Section for details) . All reported spectra were background-subtracted. Raman spectra of red pen inks are reported in **Figure 3(a)**. Readable but low intensity spectra were obtained for all pens, except Medium red for which a featureless spectrum saturated by fluorescence was obtained. The generation of high fluorescence interference was not surprising as red colored inks are characterized by strong absorption close to the selected excitation wavelength, which often results in concomitant generation of interference fluorescence emission, and consequent masking of Raman signals.[25] The spectra of Fine Crystal, Fine and Medium Crystal showed similar spectral features, suggesting the presence of a common main dye component initially identified as rhodamine B (Basic Violet 10, C.I. 45170) from diagnostic bands at 1647 and 1505 cm^{-1}. The spectrum of rhodamine B, also reported in **Figure 3(a)** for comparison, supported this preliminary attribution. Raman spectra of Crystal pink and Crystal purple pens (**Figure 3(c)**) were featureless and characterized by strong fluorescence interference. Raman spectra of green pens (**Figure 3(e)**) displayed clear features different from each other except for Fine and Medium Crystal, which showed similar spectral features, suggesting very similar dye chemical composition. Specifically, Fine and Medium Crystal green pens showed bands centered at 1599 (quadrant stretching mode of the phenyl ring), 1501 and 1410 (C=C pyrazole bending and the C-H bending mode of phenyl rings) and 482 cm^{-1} overlapping with main bands of yellow tartrazine reference dye (Acid Yellow 23, AY23, C.I. 19140) whose spectrum is also reported in **Figure 3(e)** for comparison.[33] Green Crystal ink showed spectral bands centered at 1587, 1478, 1409, 492 cm^{-1} which could not be identified. Medium green displayed bands at 1458, 1434, 1401 and 1138 cm^{-1} which could not be identified.

In order to further identify dye pen composition, SERS spectra were also recorded at 514 excitation wavelength.

Figure 3(b,d,f) show SERS spectra for the three sets of pens, in general displaying strongly enhanced features compared to Raman spectra especially evident in red, pink and purple pens. All five red BIC ballpoint pens showed similar spectral features attributable to rhodamine B, which SERS spectrum is also reported in **Figure 3(b)** for comparison. Specifically, vibrational bands were observed at 1653 (C–C bending and C=C stretching of xanthene aromatic ring), 1532-1508 (aromatic C–H bending), 1357-1281 (aromatic C–C bending), and 621 (xanthene ring puckering) cm^{-1}.[34] Rhodamine B and/or rhodamine 6G had been previously identified in red ballpoint pen formulations through easy ambient sonic spray ionization mass spectrometry (EASI-MS) analysis[35,36] and LDI-TOF-MS.[37] Interestingly, while clear distinction between rhodamine B and rhodamine 6G could not been achieved with mass spectrometry techniques, the reported SERS analysis clearly showed presence of rhodamine B in red ink formulations. SERS spectra of Crystal pink and Crystal purple pens (**Figure 3(d)**) also displayed spectral features similar to each other and similar to the red pens (main bands at 1650, 1505 and 1360 and 622 cm^{-1}), therefore also suggesting presence of rhodamine B in the ink mixture. However, closer observation of Crystal purple SERS spectrum revealed the presence of small additional bands centered at 914 and 420 cm^{-1}, indicative of the presence of an additional dye in the ink mixture. Because of the intense purple color of the ink, and its occurrence in blue and black BIC pen ink formulations, the presence of try-arylmethene dye crystal violet (Methyl violet 10B, CI 42555) was hypothesized.[11,38] The SERS spectrum of crystal violet is also reported in **Figure 3(d)** and showed multiple band overlaps (1616, 1589, 1567, 1182, 913, 415 cm^{-1}) with the crystal purple SERS spectrum. Bands at 1616 and 1589 cm^{-1} were assigned to the stretching of benzene rings, band at 1182 and 913 cm^{-1} were associated to asymmetric stretching and bending of C-C_{center}-C

bonds whereas the band at 415 cm^{-1} was associated to bending of the C-N-N bonds.[39] The presence of crystal violet in the crystal purple pen formulation was also evidenced by SERS spectrum taken at 532 nm and reported in the **Figure S3**. At the above wavelength bands clearly attributable to crystal violet were found at 1619, 1587, 1530, 1176, 910, 803 and 725 cm^{-1}. In addition, crystal purple showed bands at 1646, 1509 and 768 cm^{-1} assigned to rhodamine B, thus further confirming its occurrence in the ink mixture. The SERS spectra of green pens (**Figure 3(f)**) did not show strong enhancement compared to the Raman spectra. The presence of tartrazine hypothesized through Raman measurements in Fine and Medium crystal inks could also be hypothesized by SERS analysis because of the overlapping of bands at 1602 and 485 cm^{-1}. In addition SERS spectra of Fine, Medium Crystal and Medium showed a band at 1339 cm^{-1} whereas Crystal and Medium showed a band at 1526 cm^{-1}, which were both attributed to internal vibrations of copper phthalocyanine (CuPh) macrocycle.[40] From previous work carried out in our group, it is known that green inks are often constituted by a mixture of blue and yellow dyes and that blue phthalocyanine Solvent Blue 38 pigment (C.I. 74180) is used in BIC pen formulations.[27] Therefore, presence of CuPh blue 38 was hypothesized. Clearer indication of Blue 38 occurrence in green pens was obtained by SERS analysis at 532 nm (see **Figure S3**) where Medium, Crystal and Medium crystal green pens showed diagnostic bands at 1541, 1453, 1341 and 712 cm^{-1}. These results were in reasonable in agreement with literature data reporting presence of halogenated CuPh (Pigment Green 36) in green ink Uniball pen formulations by laser desorption mass spectroscopy (LDMS) analysis.[41]

In order to confirm results and also to test the capabilities of handheld instrumentation for the analysis of inks on paper, Raman and SERS measurements were also carried out at illumination of 785 nm with the use of Raman handheld instrumentation (**Figure 4*)**. No readable Raman spectra were obtained by handheld illumination of colored paper and therefore only results of

SERS analysis are reported. In addition, the initial SERS spectra of red/pink/purple pen inks overlapped each other and showed bands mainly attributable to the paper background (data not shown). In order to increase sensitivity, a hydrolysis step was introduced, whereby a droplet of HCl (2 μL, 1 M) was deposited on the pen written area on paper 24 hr prior Ag nanopaste deposition and SERS measurement. It has been reported that this pre-treatment step significantly improves SERS signals, by promoting the formation of Ag+_ halide complexes and therefore facilitating adsorption of the chromophores to the metal surface.[42] In order to facilitate attribution, reference samples deposited on paper substrates were subjected to the same treatment. The SERS spectra of all red pen spectra (**Figure 4(a)**) showed diagnostic bands of rhodamine B at 1655, 1518, 1365, 1292, 1204, 1988 and 633 cm^{-1}, confirming the attribution previously made by the use of bench instrumentation.

The SERS spectrum of Crystal pink pen reported in **Figure 4(b)**, overlapped with the spectra of the red pens, therefore also confirming the presence of rhodamine B. The SERS spectrum of Crystal purple clearly showed spectral features of rodhamine B and crystal violet. Specifically, the crystal purple pen showed bands at 1525 and 1286 cm^{-1} clearly assigned to rodhamine B and bands at 1613, 1579, 1380, 1172, 907, 800, 528 and 423 cm^{-1} associated with crystal violet. In contrast, spectra of green inks could be acquired without use of HCl treatment. The SERS spectra of green pens (**Figure 4(c)**) showed evidence of blue 38 presence in all pens from bands centered at 1536, 1337, 752 and 726 cm^{-1}. For comparison also spectra from rhodamine B, crystal violet, blue 38 and tartrazine references were reported in **Figure 4**.

In order to verify results additional spectral analysis was carried out whereby the UV-vis spectra of pen inks dissolved in MeOH were compared with spectra of reference dyes identified during Raman/SERS analysis. **Figure 5(a)** shows that spectra of all red dyes were equivalent to each other and showed a maximum band centered at 554 nm, which overlapped the absorption

spectrum of rodhamine B. Interestingly, all red pen UV-vis spectra also showed maxima centered at 416 nm, attributable to a yellow component which remains unidentified. The UV-vis absorption spectra of pink and purple Crystal pen inks (**Figure 5(b)**) also overlapped with the spectrum of rhodamine B, displaying a maximum at 554 nm. However, the purple Crystal ink showed an additional shoulder at 588 nm, which well overlapped with the absorption band of crystal violet dye. A small additional band at 663 was also observed and attributed to a phthalocyanine compound, possibly blue 38. The presence of blue38 could also be proved by close inspection of SERS spectra taken at 785 nm excitation wavelength, where two bands at 1340 and 750 cm^{-1} were observed, which overlapped with an equivalent band of blue 38 at that excitation wavelength (see **Figure S4**). Finally, the UV-vis spectral analysis of green pen inks confirmed results of the Raman/SERS analysis and revealed the presence of a blue component (blue 38) centered at 665 nm for all pens. A yellow component (possibly tartrazine) was also identified from a band centered at 430 nm in Fine, Medium crystal and Medium pens. Crystal pen showed a large band at 430 nm which overlapped the band of tartrazine. However, as no evidence of tartrazine was found by Raman/SERS analysis, we conclude that a yellow component molecularly similar to tartrazine should be present in this formulation. Additional bands at 622 and 692 nm were found for Medium and Crystal green inks, respectively. Such bands were associated to the presence of additional unidentified blue components.

Further characterization was carried out by Thin Layer Chromatography (TLC). Results were consistent with data obtained with Raman, SERS and UV-vis spectroscopies and are reported in **Figure S5**.

Table 2 shows a summary of the chemical composition information that was achieved with each applied analytical technique and under different experimental conditions (for example different wavelength illuminations used for Raman and SERS analysis). It is interesting to show that

although Raman would be the analytical tool of choice due to its fingerprinting capabilities and non-invasiveness, its effectiveness for the identification of dyes in red colored inks at 514 nm excitation wavelength was limited by the strong fluorescence interference generating from excitation in close proximity to the main dye component rodhamine B molecular absorption resonance (absorbance maximum 554 nm). Therefore identification of rhodamine B was possible for three red inks out of four and no identification was possible for pink and purple inks. No Raman response was obtained for red, pink and purple colored dyes at the other tested excitation wavelengths (532 and 785 nm). Identification of green inks was also difficult at 514 nm, as only tartrazine could be detected for Fine and Medium Crystal green inks. Better results were obtained at 532 and 785 nm, with detection of tartrazine and blue 38 possible in three out of four analyzed green inks. Therefore the application of Raman spectroscopy would require the use of multi-line excitation systems to gather complete chemical compositional information.

On the other hand, SERS analysis at 514 nm excitation was very effective for the characterization of red, pink and purple inks. The enhancement was attributed to the combination of three effects: i) an electromagnetic effect (EM), due to the use of illumination wavelength in plasmonic resonance with the Ag nanopaste (see **Figure S1**); ii) Surface Enhanced Resonance Raman Spectroscopy (SERRS) condition (excitation wavelength in molecular resonance with the main dye component); iii) possible chemical enhancement (CE) processes, due to electrostatic interaction between positively charged rhodamine B and negatively charged Ag nanopaste. SERS was also effective for identification of green ink mixtures due to EM plasmonic enhancement processes. However, the complete identification of blue 38 in green Fine and Medium crystal inks still required the use of an additional excitation lines (either 532 nm or 785 nm), possibly due to the low concentration of this component in the lighter hues green inks.

The use of handheld instrumentation in combination with SERS resulted very effective in identification of dye components for all inks. This result is extremely relevant for the practical implementation of SERS in real analysis where portable instrumentation is often mandatory. The successful generation of SERS signals for red, pink and purple inks was ascribed to a combination of effective accumulation of analyte on the plasmonic surface (hydrolysis process occurring on deposited Ag nanopaste) and effective quenching of fluorescence from the deposited Ag nanopaste.

Interestingly, more dye components were identified by the use of complementary techniques UV-vis and TLC. Specifically, the presence of a yellow component additional to rhodamine B in red inks was only revealed by UV-vis and explained the difference in color between Crystal pink (pure rhodamine B) and red inks. Additional blue components were revealed by UV-vis for Medium and Crystal green. TLC was also used to support results. TLC confirmed the presence of an additional component in red pens which appeared of orange coloration for Fine Crystal, Fine and Medium Crystal and yellow for Medium. Yellow spots not attributable to tartrazine were obtained for Medium and Crystal green inks.

The information collected by this analysis could nicely complement analytical information more widely available for blue and black inks and could constitute the basis for the construction of a spectral database for writing inks. This in turn could support the analysis of real pen artworks. In order to show a practical example, a case study is shown where two drawings made by red and green BIC ballpoint pens and donated by French artist Anne-Flore Cabanis were analyzed by Raman spectroscopy.

Figure. 6(a,b) show photographs of the drawings made by a continuous line bent at 90 degree angles in order to achieve an overall circular pattern. Careful observation of the drawings under optical microscope (**Figure 6(b,c)**) showed slightly different color intensities and areas of high

ink accumulation generated by the occasional harder pressure of the pen on the paper at resting and corner points, respectively. As result, some paper fibers showed stronger ink accumulation, where Raman spectroscopy was carried out in order to obtain maximized response. The Raman spectrum of the red line (**Figure 6(d)**) showed bands at 1360, 1506 and 1649 cm^{-1} attributable to rodhamine B. As all analyzed red pens showed the same bands of rodhamine B, identification of the exact BIC pen used by the artist was not possible only with the use of non-invasive analytical techniques. However, successful identification of the main dye component was achieved, which is relevant for conservation purposes. The Raman spectrum of the green line (**Figure 6(f)**) showed bands at 1597, 1502, 1475, 1408, 1341, 1000, 808, 769, 482 cm^{-1} and strong overlap with spectral features of green Fine BIC pen previously analyzed. In this case identification was made easier by the diversification in chemical composition of analyzed pens. This information is relevant for the development of future conservation protocols and for detection of forgeries/attribution of originality in pen artworks.

4. Conclusion

Raman spectroscopy and SERS were applied for the identification of dye mixtures in commercial colored (red, pink, purple and green) BIC ballpoint pens. All pens inks were analyzed as colored areas on commercial paper. The use of laser excitation wavelengths at 514, 532 and 785 nm allowed Raman identification of main dyes for all pens. The great advantage of Raman spectroscopy relied in its non-invasive nature. However, more efficient identification was achieved by SERS, through deposition of Ag nanopaste on colored paper. Enhanced Raman signals were in general achieved for each selected illumination wavelength, thus not requiring the use of multiple illumination lines. The use of SERS was particularly effective for the characterization of red, pink and purple inks, whose Raman spectra were characterized by high fluorescence backgrounds. The drawback of SERS relies in its invasive nature, associated to the

deposition of nanopaste on the analytical surface. This often prevents the direct applicability of SERS to art objects. Nevertheless, this works proves that SERS can be an effective tool for analysis of microextracted objects and other artistic media such as pens and felt-tip pens.

Handheld Raman instrumentation was also successfully applied for analysis of inks in combination with SERS. This method also required the use of invasive procedures (hydrolysis and nanopaste deposition). The hydrolysis step did not cause further damage to the paper substrate as the area treated with HCl was the same area subsequently covered by the nanopaste. The advantage of this method relied on the use of low cost, easy to operate and easily deployable instrumentation. Finally, data collected by Raman spectroscopy and SERS were complemented by UV-vis spectroscopy and TLC which confirmed results obtained and allowed identification of additional dye components in red and green inks. Although data in this work were mainly obtained by the use of invasive methods, this work greatly complements data already obtained for colored pens by other methods and therefore is relevant for the construction of an initial spectral database of ballpoint pen inks. The construction of such database would benefit from further investigation. However, BIC pens are sold in six types of point and sixteen colors around the world. Having analyzed three shades of green and one of red, purple and pink (one shade of blue was analyzed in a previous publication) we feel that the number of analyzed colors is representative of what is used in reality by artists. Due to the large amount of ink-based artworks and their light sensitivity, such database will be useful for the characterization of ink-based artworks which in turn will inform the development of conservation strategies tailored to the preservation of these light fugitive art objects.

Supporting Information

Supporting Information is available.

Acknowledgements

The authors wish to thank artist Anne-Flore Cabanis for her generous donation of the pen artworks. This work was supported by the European Union H2020 project Nanorestart (646063).

References

[1]. B. Genocchio To See the World in Ballpoint Pen., *The New York Times*. Manhattan, New York, USA, **2017.**

[2]. J. A. Siegel, P. J. Saukko. *Encyclopedia of Forensic Sciences, Academic Press*, Waltham, 2nd edn, **2013**, 375-379.

[3]. R. A. Merrill, E. G. Bartick. *J. Forens Sci.* **1992**, 37:528-541.

[4]. S. Bell, *Encyclopedia of Forensic Sciences*, Infobase Publishing, New York, **2008**.

[5]. F. C. Izzo, V. Vitale, C. Fabbro, H. Van Keulen, *Microchem. J.* **2016**,*124*, 919-928.

[6]. R. W. Jones, J. F. McClelland, *Forensic Sci. Int.* **2013**, *231*, 73-81.

[7]. L. Nguyen, M. Moini, *Forensic Chem.*, **2016**, *1*, 78-85.

[8]. L. Brazeau, M. Gaudreau, *J. Forensic Sci*. **2007**, *52*, 209-215.

[9]. V. S. Amador, H. V. Pereira, M. M. Sena, R. Augusti, E. Piccin, *J. Am. Soc. Mass Spectr.* **2017**, *28*, 1965-1976.

[10]. C. Zaffino, A. Passaretti, G. Poldi, M. Fratelli, A. Tibiletti, R. Bestetti, I. Saccani, V. Guglielmi, S. Bruni, *J. Cult. Herit.* **2017**; *23*, 87-97.

[11]. A. Alyami, D. Saviello, M. A. P. McAuliffe; A. Mirable; L. Lewis, D. Iacopino *PhysChemChemPhys*, **2017**, *19*, 14652-14658.

[12]. J. Zieçba-Palus, M. Kunichi, *Forens. Sci. Int.* **2006**, *158*, 164-172.

[13]. W. Dirwono, J. S. Park, M. R. Augustin-Camacho, J. Kim, H. M. Park, Y. Lee, K. B. Lee, *Forensic Sci. Int*. **2010**, *199*, 6-8.

[14]. S. Dhara, N. L. Misra, S. D. Maind, S. A. Kumar, N. Chattopadhyay, S. K. Aggarwal, *Spectrochim. Acta B* **2010**, *65*, 167-170.

[15]. A. Braz, M. López- López, C. García-Ruiz, *Forensic Sci. Int.* **2013**, *232*, 206-212.

[16]. I. Geiman, M. Leona, J. R. Lombardi, *Forensic Sci.* **2009**;*54*, 947-952.

[17]. F. Casadio, C. Daher, L. Bellot-Gurlet, *Top Curr. Chem.* **2016**, *374*, 62.

[18]. J. R. Lombardi, M. Leona, T. Vo-Dinh, P. Antoci U.S.D. Justice, Editor. National Institute of Justice, **2009**.

[19]. N. D. Israelsen, C. Hanson, E. Vargis, *Scient. World J.* **2015**, *2015*, 124582-124594.

[20]. K. A. Willets, R. P. Van Duyne, *Ann. Rev. Phys. Chem.* **2007**, *58*, 267-97.

[21]. B. Sharma, R. R. Frontiera, A. I. Henry, E. Ringe, A. P. Van Duyne, *Mater. Today* **2012**, *15*, 16-25.

[22]. F. Pozzi., J. R. Lombardi, S. Bruni, M. Leona, *Anal. Chem.* **2012**, *84*, 3751-3757.

[23]. K. L. Wustholz, C. L. Brosseau., F. Casadio, R. P. Van Duyne, *Phys.Chem.Chem.Phys*. **2009**, *11*, 7350-7359.

[24]. A. V. Whitney, R. P. Van Duyne., F. Casadio, *J. Raman Spectrosc.* **2006**, *37*, 993-1002.

[25]. A. Cesaratto, J. R. Lombardi, M. Leona, *J. Raman Spectrosc.* **2017**, *48*, 418-424.

[26]. F. Pozzi, M. Leona, *J. Raman Spectrosc.* **2016**, *47*, 67-77.

[27]. D. Saviello, A. Alyami, M. Trabace, A. Mirabile, R. Giorgi, P. Baglioni, D. Iacopino, *RSC Adv.* **2018**, *8*, 8365-8371.

[28]. D. Saviello, A. DiGioia, P. Turenne, M. Trabace, R. Giorgi, A. Mirabile, P. Baglioni, D. Iacopino, *J. Raman Spectrosc*. **2018**, 10.1002/jrs.5411.

[29]. D. Saviello, M. Trabace, A. Alyami, A. Mirabile, R. Giorgi, P. Baglioni, D. Iacopino, *Talanta* **2018**, *181*, 448-453.

[30]. L. Polavarapu; A. La Porta, S. N. Novikov, M. Coronado-Purchau, L.M Liz-Marzán. *Small* **2014**, 15, 3065-3071.

[31]. R. M. Seifar, J. M. Verheul, F. Ariese, U. A. T. Brinkman, C. Gooijer, *Analyst* **2001**, *126*, 1418-1422.

[32]. P. C. White, *Sci Justice* **2003**, 43, 149-152.

[33]. G. Germinario, S. Garrappa, V. D'Ambrosio; I. D. van der Werf, L. Sabbatini, *Anal. Bioanal. Chem.* **2018**;*410*, 1079-1094.

[34]. S. Lin, W. L. J. Hasi, L. Xiang, S. Han, X. Lou, F. Yang, D. Y. Lin, Z. W. Lu, *Anal. Methods* **2015**, *7*, 5289-5294.

[35]. P. M. Lalli, G. B. Sanvido, J. S. Garcia, R. Haddad, R. G. Cosso, D. R. J. Maia, J. J. Zacca, A. O. Maldarnec, M. N. Eberlin, *Analyst* **2012**, *135,* 525-532.

[36]. H. S. Chen, *Forensic Sci. J.* **2005**, *4*, 15-27.

[37]. J. D. Dunn, J. A. Siegel; J. Allison, *J. Forensic Sci.* **2003**, *48*, 652-657.

[38]. J. D. Dunn, J. Allison, *J. Forensic Sci.* **2007**, *52*, 1205-1211.

[39]. M. V. Canamares, C. Chenal, R. L. Birke, J. R. Lombardi, *J. Phys. Chem. C* **2008**, 112, 20295-20300.

[40]. D. Li, Z. Peng, L. Deng, Y. Shen, Y. Zhou, *Vibr. Spectroc.* **2005**, *39*, 191-199.

[41]. K. Papson, S. Stachura; L. Boralsky, J. Allison, *J. Forensic Sci.* **2008**, 53, 100-106.

[42]. A. Otto, A. Bruckbauer, Y. X. Chen, *J. Molec. Stru*. **2003**, *661-662*, 501-514.

Figures and Tables

Table 1. List of BIC pens investigated in this work.

Name	Color
Fine	Red
Medium crystal	Red
Fine crystal	Dark red
Medium	Light red
Crystal	Pink
Crystal	Purple
Fine	Dark green
Medium crystal	Dark green
Crystal	Light green
Medium	Green

Table 2. List of dye components identified by Raman, SERS, UV-vis and TLC analysis in all analyzed pen inks.

Pen	514 Raman	532 Raman	785 Raman bench
Fine crystal red	Rhodamine B		
Fine red	Rhodamine B		
Medium crystal red	Rhodamine B		
Medium red			
Crystal pink			
Crystal purple			
Medium green		Blue 38, tartrazine	Blue 38
Crystal green			
Fine green	Tartrazine	Blue 38, Tartrazine	Blue 38
Medium crystal green	Tartrazine	Blue 38, Tartrazine	Blue 38

Pen	514 SERS	532 SERS	785 SERS bench	785 SERS HH
Fine crystal red	Rhodamine B	Rhodamine B		Rhodamine B
Fine red	Rhodamine B	Rhodamine B		Rhodamine B
Medium crystal red	Rhodamine B	Rhodamine B		Rhodamine B
Medium red	Rhodamine B	Rhodamine B		Rhodamine B
Crystal pink	Rhodamine B	Rhodamine B		Rhodamine B
Crystal purple	Rhodamine B, crystal violet	Rhodamine B, crystal violet	Rhodamine B, crystal violet, blue 38	Rhodamine B, crystal violet
Medium green	Blue 38, tartrazine	Blue 38	Blue 38	Blue 38
Crystal green	Blue 38			Blue 38
Fine green	Tartrazine	Blue 38	Blue 38	Blue 38
Medium crystal green	Tartrazine, blue 38	Blue 38	Blue 38	Blue 38

Pen	UV-vis	TLC
Fine Crystal red	Rhodamine B + orange band	Rhodamine B + orange spot
Fine red	Rhodamine B + orange band	Rhodamine B + orange spot
Medium Crystal red	Rhodamine B + orange band	Rhodamine B + orange spot
Medium red	Rhodamine B + orange band	Rhodamine B+ yellow spot
Crystal pink	Rhodamine B	Rhodamine B
Crystal purple	Rhodamine B, crystal violet, blue 38	Rhodamine B, crystal violet, blue 38
Medium green	Blue 38, tartrazine	Blue 38, yellow spot
Crystal green	Blue 38 + blue +yellow band	Blue 38, yellow spot
Fine green	Blue 38, tartrazine	Blue 38, tartrazine
Medium Crystal green	Blue 38 + blue, tatrazine	Blue 38, tartrazine

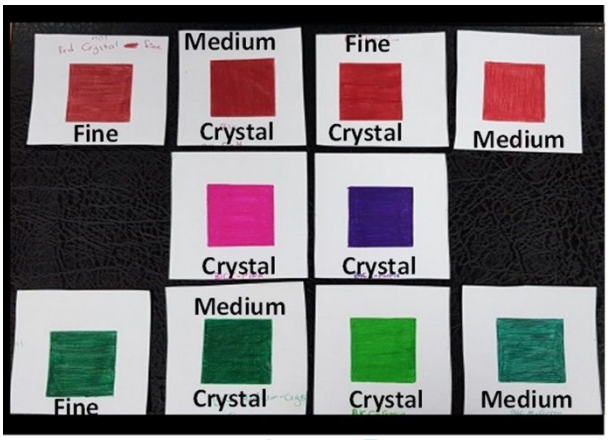


Figure 1. Photographs of colored squares drawn on commercial paper with selected BIC pens.

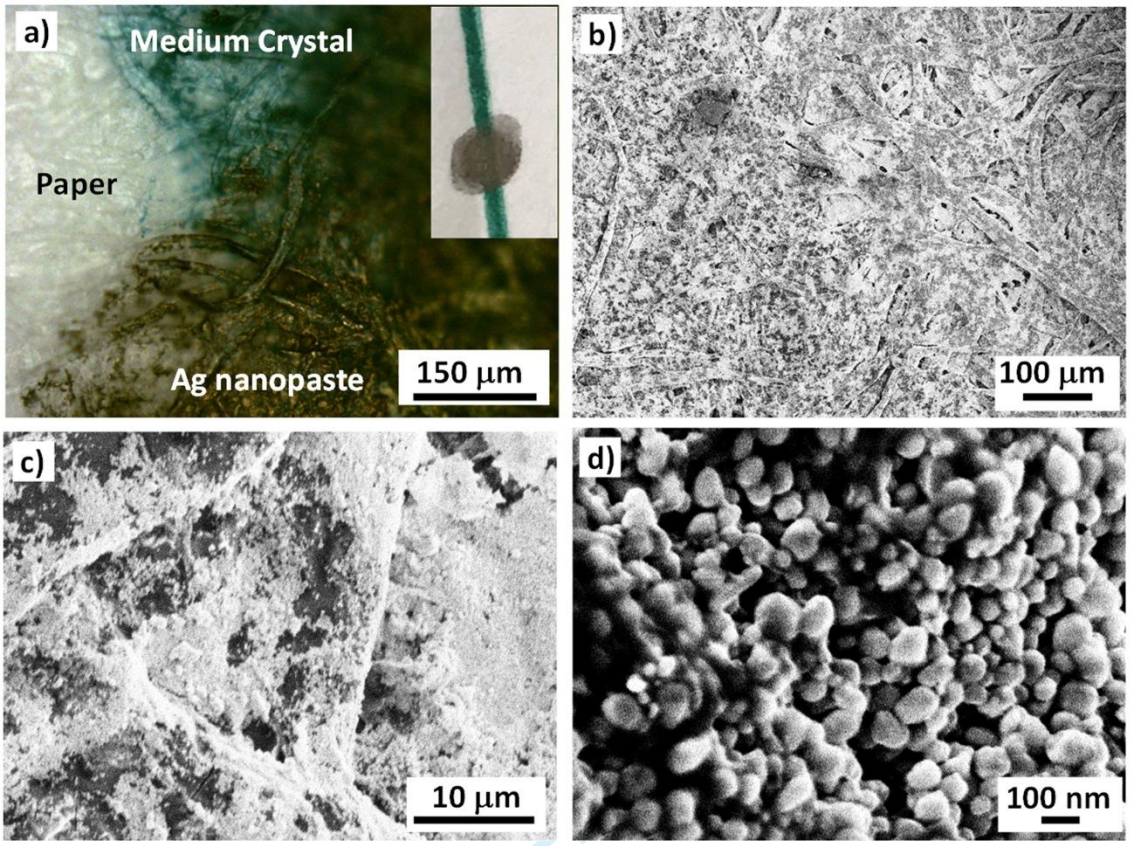


Figure 2. **a** Optical microscopy image of green Medium Crystal line drawn on paper with deposited Ag nanopaste. Low (**b**), medium © and high (**d**) magnification SEM images of Ag nanopaste deposited on green colored paper showed in (**a**).

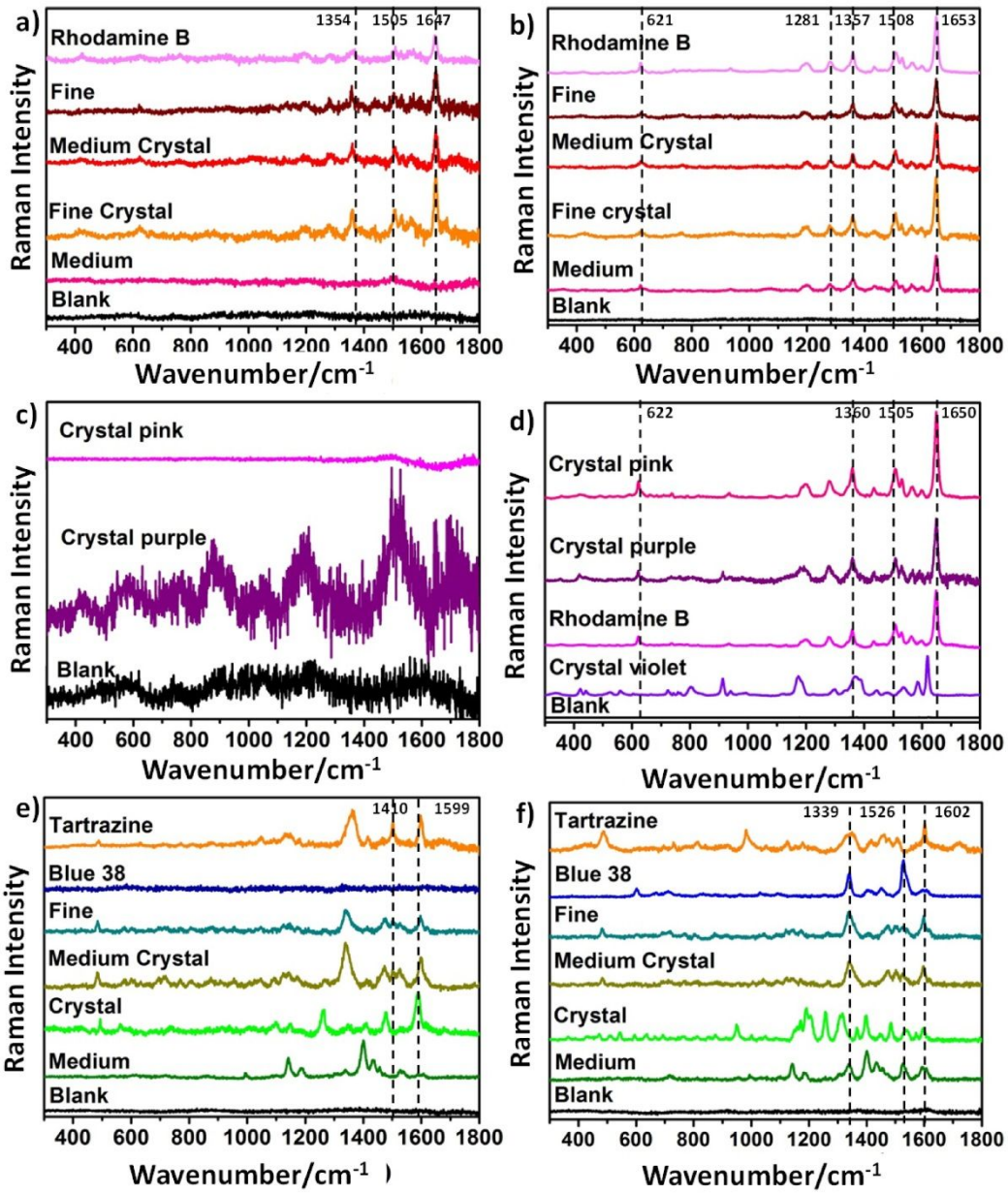


Figure 3. Raman (**a**) and SERS (**b**) spectra of red pen inks and reference spectra of rhodamine B. Raman (**c**) and SERS (**d**) spectra of pink and purple Crystal pen inks and reference spectra of rhodamine B and crystal violet. Raman (**e**) and SERS (**f**) spectra of green pen inks and reference spectra of tartrazine and blue 38. All spectra were recorded at 514 nm excitation wavelength and were background subtracted.

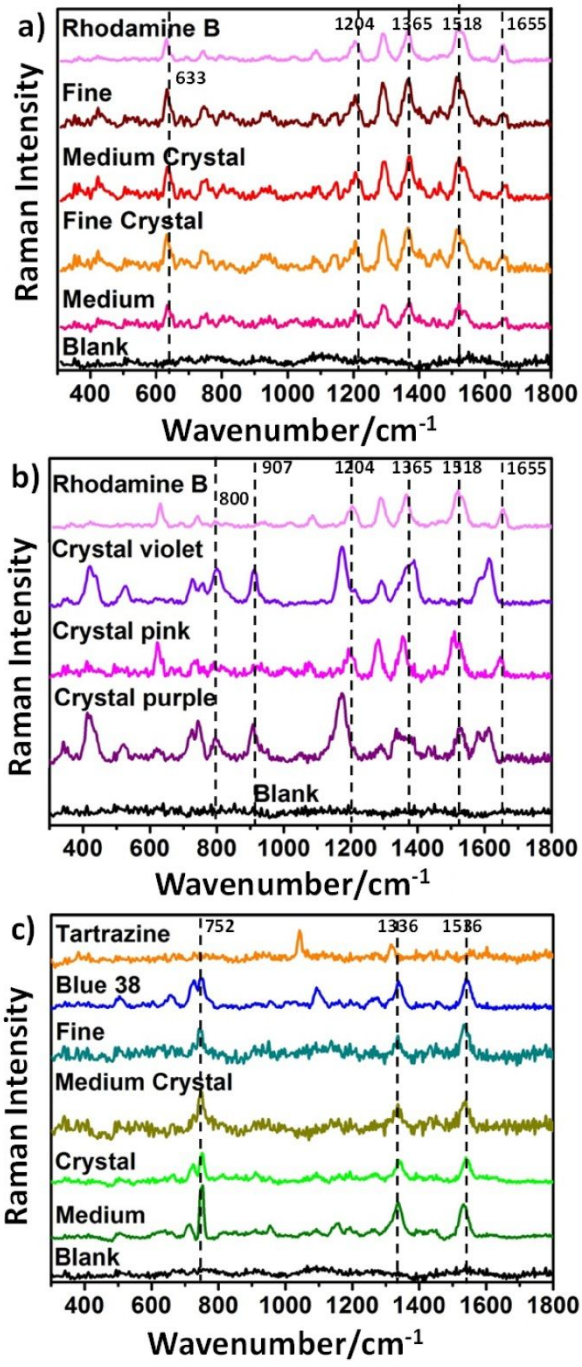


Figure 4. SERS spectra of BIC ballpoint pen inks (**a**) red, (**b**) pink and purple, (**c**) green. All spectra were taken with handheld instrumentation at 785 nm excitation wavelength.

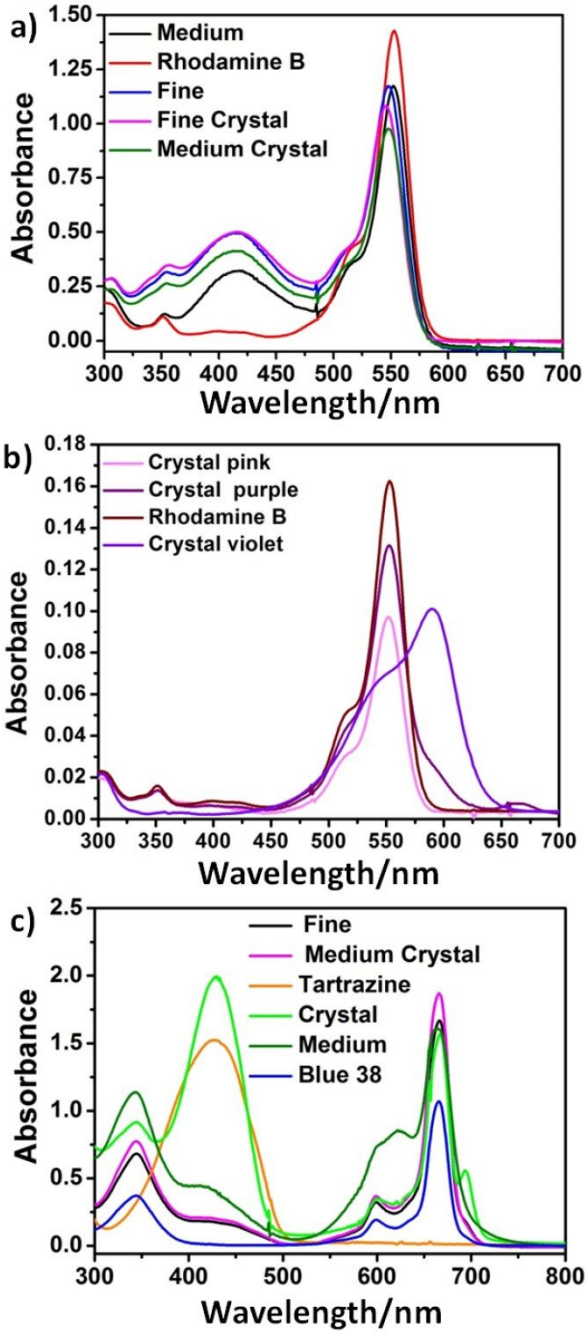


Figure 5. UV-vis spectra of BIC ballpoint pen inks and reference dyes dissolved in MeOH: (**a**) red pens and rhodamine B, (**b**) pink, purple inks and rodhamine B, crystal violet references, (**c**) green inks and tartrazine, blue 38 references.

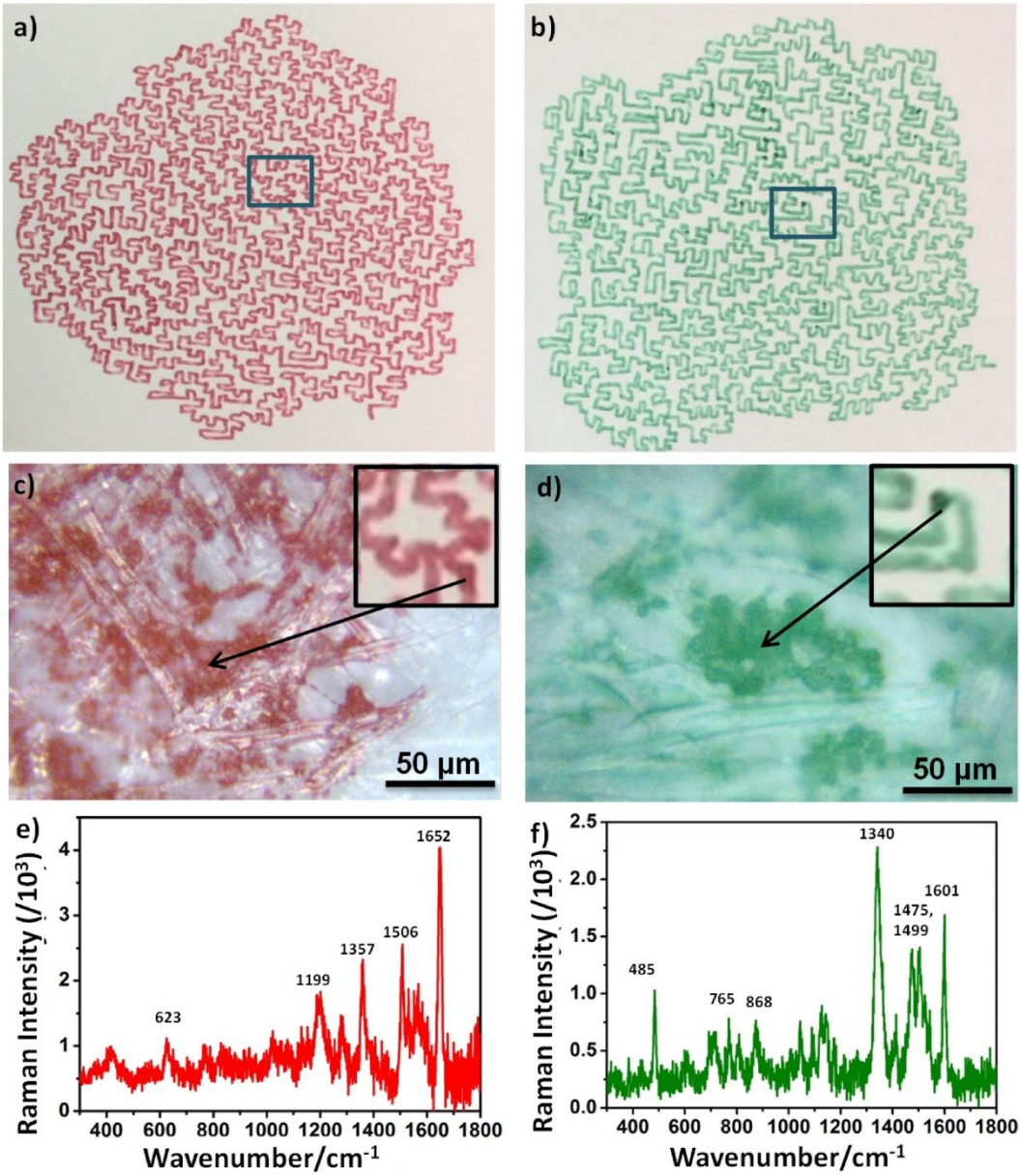
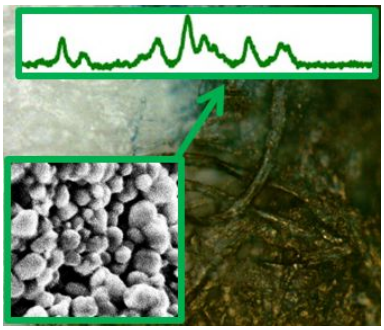


Figure 6 (**a,b)** Photographs of Anne-Flore Cabanis drawings made by red and green ballpoint pens. **C,d** optical microscopy images of Anne-Flore Cabanis drawings. (**e, f**) Raman spectra of red and green pen Anne-Flore Cabanis drawings.

Table of Contents Entry

Raman spectroscopy and SERS was used for the identification of dye content in commercial coloured BIC ballpoint pens. SERS was enabled by deposition of Ag nanopastes on analytical surfaces. Compared to Raman spectroscopy, SERS provided enhanced spectral features and efficient quenching of fluorescence necessary for the unequivocal identification of dye mixtures in all analysed pens.



Identification of Dye Content in Colored BIC Ballpoint Pen Inks by Raman Spectroscopy and Surface Enhanced Raman Scattering (SERS)

A. Alyami, K. Barton, L. Lewis, A. Mirabile and D. Iacopino*